REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 1, 18, 44, 47, 67, 71, 74, 78, 81-85, 90, and 92-93 are amended, claim 89 is canceled, and claims 95-96 are added. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the claims prior to amendment, which claims are present in a continuation of the above-referenced application. Claims 1, 3-6, 9, 11-12, 15, 18, 20-21, 24-39, 41-45, 47, 60, 64, 67, 69-71, 74, 76-78, 80-88, and 90-96 are pending.

The 35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner rejected claims 1, 3-6, 9, 11-12, 15, 18, 20-21, 24-39, 41-45, 47, 60, 67, 69-71, 74, 76-78, 80-83, and 85-94 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Examiner asserts that the phrase "a reduced number of a combination of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and/or promoter sequences" is indefinite because 1) such a calculation is impossible, 2) the scope of the claims would change as new members of transcription factor binding sequences are recognized, and 3) the terms define a group of sequences by function and although the art defines some specific sequences in each group, many other sequences have the same function and may not be taught or known to the art. This rejection is respectfully traversed.

As discussed in the Amendment filed on September 22, 2005, it is Applicant's position that the phrases "transcription factor binding sequences," "intron splice sites," "poly(A) addition sites" and prokaryotic 5' noncoding regulatory sequences such as "promoter sequences" are conventionally used and understood by the art. See, e.g., U.S. Patent No. 5,670,356 ("transcription factor binding sites"), WO 97/47358 ("intron splice sites"), Iannaconne et al., Plant Mol. Biol., 34:485 (1997) ("polyA sequences"), and Pan et al., Nucl. Acids Res., 27:1094 (1999) ("prokaryotic promoters," "poly(A) signals," and "exon-intron boundaries") (of record), and Faisst and Meyer, Nucl. Acids Res., 20:3 (1992) (cited at page 50, lines 6-7 of Applicant's specification), which discloses a compilation of vertebrate encoded transcription factors. At

page 4 of the Office Action dated December 19, 2005, the Examiner acknowledges that those terms are conventional in the art. Therefore, one of skill in the art would understand the metes and bounds of "mammalian transcription factor binding sequences," "intron splice sites," "poly(A) addition sites" and "prokaryotic 5' noncoding regulatory sequences."

With regard to calculating the number of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, the Examiner is requested to consider that Example 1 in the above-identified specification discloses that synthetic click beetle luciferase sequences were prepared that had a reduced number of a combination of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences. For instance, it is disclosed that mammalian codon replacement in a parent click beetle luciferase sequence yielded a mammalian codon optimized click beetle luciferase sequence (GRver1). Removal of intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, e.g., promoter sequences, in the mammalian codon optimized click beetle luciferase sequence by codon replacement, resulted in a sequence, GRver2, that had about 100 mammalian transcription factor binding sequences. Replacement of codons in GRver2 to remove those mammalian transcription factor binding sequences, and intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, yielded a sequence, GRver3, that had about 50 newly introduced mammalian transcription factor binding sequences. Replacement of codons in GRver3 to remove those mammalian transcription factor binding sequences, and intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, yielded a sequence, GRver4, that had about 20 newly introduced mammalian transcription factor binding sequences. Those newly introduced mammalian transcription factor binding sequences were removed by codon replacement to yield GRver5. It is disclosed that GRver5 was devoid of eukaryotic transcription factor binding sequences but did contain one splice acceptor sequence.

Moreover, as described in the Rule 132 Declaration enclosed herewith and executed by Monika Wood, a co-inventor of the above-referenced application, using software and a database that are available to the public and comparable to those disclosed in the application, she determined the number of mammalian transcription factor binding sequences in *luc+*, a sequence

described in Sherf et al. (U.S. Patent No. 5,670,356), a reference cited against the claims under 35 U.S.C. § 103(a).

Thus, contrary to the Examiner's assertion, the calculation of the number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences in a particular sequence is possible.

With regard to the alleged change in scope of claims which recite mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences, it is Applicant's position that intron splice sites, poly(A) addition sites, and prokaryotic 5' noncoding regulatory sequences represent relatively conserved sequences that were well known prior to Applicant's effective filing date (Mount, Am. J. Hum. Genet., 67:788 (2000) (consensus and other conserved splice sites), Jensen et al., Appl. Environ. Microbiol., 64:82 (1998) (synthetic promoters with known consensus sequences, see abstract), Hsieh et al., J. Bacteriol., 177:5740 (1995) ("a potential ribosome-binding site", see abstract and Figure 3; also see page 5742 and Figure 3 for conserved promoter sequence motifs "-10" and "-35"), and Andrews et al., J. Virol., 67:7705 (1993) ("a canonical poly(A) consensus signal"; see abstract), a copy of each is enclosed herewith). And although there may be new members added to the group "mammalian transcription factors" over time, the independent claims in the present application provide that the synthetic nucleic acid molecules have a reduced number of a combination of transcription factor binding sequences, as a result of codon replacement with mammalian high usage codons and mammalian codons that are not high usage.

With regard to "SEQ ID NO:22" (a synthetic *Renilla* luciferase sequence) in claim 47, the Examiner is requested to consider Applicant's election of species in the Response to Restriction Requirement dated November 12, 2002, in which a click beetle luciferase sequence was elected. Nevertheless, claim 47 is amended.

The amendments to claim 90 and 93 address the § 112, second paragraph, rejection of those claims at pages 5-6 of the Office Action.

Accordingly, withdrawal of the 35 U.S.C. § 112, second paragraph, rejections is respectfully requested.

The 35 U.S.C. § 112, First Paragraph, Rejections

The Examiner rejected claims 1, 3-6, 9, 11-12, 15, 20-21, 24-33, 35-39, 41-45, 47, 60, 67, 69-70, 81-83, and 85-94 under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for (1) a variant of a parent DNA molecule encoding a reporter polypeptide identical to a reporter polypeptide encoded by said parent DNA, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences than a mammalian codon optimized variant of the parent nucleic acid, (2) a variant of a parent DNA molecule encoding a luciferase having 90% identity to the polypeptide encoded by SEQ ID NO:2 and having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences than a mammalian codon optimized variant of SEQ ID NO:2 and (3) to any nucleic acid which will hybridize to SEQ ID NO:9 under high stringency conditions and encode a polypeptide having luciferase activity, does not reasonably provide enablement for any variant DNA molecules encoding any reporter polypeptide having at least 90% identity to a wild type reporter polypeptide, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences than a mammalian codon optimized version of the parent nucleic acid or to any nucleic acid which will hybridize to SEQ ID NO: 9 under medium stringency conditions. In particular, the Examiner aasserts that the disclosure is limited to modifying the nucleic acid sequence of a desired gene without changing the encoded protein sequence, and that it is not predictable what changes can be tolerated in an amino acid sequence and result in a desired activity. These rejections, as they may be maintained with respect to the pending claims, are respectfully traversed.

First, it is unclear how Applicant's specification teaches one of skill in the art how to make and use a variant of a parent DNA molecule encoding a reporter polypeptide identical to a reporter polypeptide encoded by said parent DNA, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences (acknowledged by the Examiner at page 6 of the Office Action) if the art worker would not recognize or understand sequences that are

mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences (see page 3 of the Office Action).

With respect to variants of reporter polypeptides, such as GFP, beetle luciferase, GUS, and CAT, as well as beta-lactamase, Applicant has provided evidence that it is well within the skill of the art to introduce substitutions into various reporter proteins and yield a variant protein with the activity of the corresponding wild-type reporter protein (see, e.g., the abstracts for Stapleton et al., Antimicrob. Agents Chemother., 43:1881 (1999); Bouthors et al., Protein Eng., 12:313 (1999); Sirot et al., Antimicrob. Agents Chemother., 41:1322 (1997); Voladri et al., J. Bacteriol., 178:7248 (1996); Murray et al., J. Mol. Biol., 254:993 (1995); and Matsumura et al., Nat. Biotechnol., 17:696 (1999), and U.S. Patent No. 5,874,304, all of record).

In particular, with regard to luciferases, numerous substitutions have been introduced into beetle luciferases without affecting the reporter property of the substitution variants (see, e.g., Kajiyama et al., Protein Engineering, 4:691 (1991)), Wood et al., J. Biolumin., 4:31 (1989), Wood et al., J. Biolumin., 5:107 (1990) and Sala-Newby et al., Biochem. J., 279:727 (1991)), U.S. Patent Nos. 5,670,356, 6,552,179, 6,387,675 and 6,602,677 (all of record). For instance, in U.S. Patent No. 6,602,677, five mutant luciferases are disclosed that have 12, 21, 32, 37 and 37 substitutions, respectively, relative to a parent luciferase. Thus, it is well within the skill of the art worker to predictably substitute amino acids in a reporter protein.

Further, the Examiner is requested to note that in Example 1 of the above-referenced application, the amino acid sequence of the click beetle luciferases encoded by synthetic nucleic acid sequences of the invention is different than the amino acid sequence of the parent click beetle luciferase. Similarly, in Example 3 of the present application the amino acid sequence of the *Renilla* luciferase encoded by a synthetic nucleic acid sequence is different than the amino acid sequence of the parent *Renilla* luciferase sequence.

With regard to the Examiner's assertion that it is not routine to screen for multiple substitutions or multiple modifications, the Examiner is requested to consider WO 99/14336, a reference cited against the claims under 35 U.S.C. § 103(a), and Arnold (Chem. Eng. Sci., 51:5091 (1996) (a copy is enclosed herewith) which disclose the introduction of multiple modifications into a nucleic acid molecule and screening for particular phenotype(s) of the encoded gene product.

Therefore, it is within the skill of the art to prepare variant proteins with a particular phenotype.

The Examiner also asserts that all variant nucleic acids that hybridize under medium stringency conditions to Applicant's synthetic nucleic acid molecules are not enabled. It is Applicant's position that one of skill in the art in possession of Applicant's specification is readily able to determine whether a variant nucleic acid molecule hybridizes under medium stringency conditions to Applicant's synthetic polynucleotides, e.g., hybridize to SEQ ID NO:9, and has an open reading frame encoding a beetle luciferase polypeptide which has at least 90% amino acid sequence identity to a luciferase encoded by a corresponding wild type nucleic acid sequence, wherein the codon composition of the open reading frame of the first polynucleotide is different at more than 25% of the codons from that of the wild type luciferase nucleic acid sequence and is different than the codon composition of a second polynucleotide which encodes a polypeptide which has at least 90% amino acid sequence identity to the polypeptide encoded by the wild type nucleic acid sequence, wherein the codons in the second polynucleotide that are different than the codons in the wild type nucleic acid sequence are mammalian high usage codons selected to result in the second polynucleotide having a reduced number of a combination of different mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites or prokaryotic 5' noncoding regulatory sequences relative to the wild type nucleic acid sequence, wherein the codons which differ in the first polypeptide relative to the second polynucleotide are mammalian codons selected to result in the open reading frame in the first polynucleotide having a reduced number of a combination of different mammalian transcription factor binding sequences, and optionally a reduced number of intron splice sites, poly(A) addition sites or prokaryotic 5' noncoding regulatory sequences, that are introduced to the second polynucleotide by selecting the mammalian high usage codons, wherein the mammalian transcription factor binding sequences are those present in a database of transcription factor binding sequences.

Claim 44 was rejected under 35 U.S.C. § 112, first paragraph. The amendments to claim 44 obviate the §112(1) rejection of that claim at page 11 of the Office Action.

Therefore, withdrawal of the § 112(1) rejections is appropriate and is respectfully requested.

The 35 U.S.C. § 103 Rejections

The Examiner rejected claims 1, 3-6, 9, 11-12, 15, 20-21, 24-39, 41-45, 60, 67, 69-70, 81, 86, and 89-94 under 35 U.S.C. § 103(a) as being unpatentable over Sherf et al. (U.S. Patent No. 5,670,356) in view of Zolotukhin et al. (U.S. Patent No. 5,874,203), Donnelly et al. (WO 97/47358), Pan et al. (Nucl. Acids Res., 27:1094 (1999)), Cornelissen et al. (U.S. Patent No. 5,952,547), and Hey et al. (U.S. Patent No. 6,169,232). The Examiner also rejected claims 47, 71, 74, 76-78, 80, 82-85, and 87-88 under 35 U.S.C. § 103(a) as being unpatentable over Sherf et al., WO 97/47358, Pan et al., Cornelissen et al., Hey et al., and further in view of Wood et al. (WO 99/14336). These rejections are respectfully traversed.

Sherf et al. disclose a synthetic firefly luciferase gene (*luc*$^+$) in which 3 internal palindromic sequences, 5 restriction endonuclease sites, 4 glycosylation sites, and 6 transcription factor binding sites that were present in the unmodified sequence were removed, and codons were altered at sequences specified in Table 2 to codons preferred ("more common") in mammalian cells, relative to a wild type firefly luciferase gene (*luc*). Of the twenty 6 to 30 bp regions which were modified, 6 regions included modifications with a dual purpose, i.e., one region was modified to eliminate a glycosylation site and a transcription factor binding site that was present in the unmodified sequence, three regions were modified to eliminate a transcription factor binding site that was present in the unmodified sequence and improve codon usage, one region was modified to eliminate two transcription factor binding sites (but not improve codon usage) that were present in the unmodified sequence, and another region was modified to improve codon usage and eliminate a restriction endonuclease recognition site.

Sherf et al. also disclose that a vector encoding Luc$^+$ or Luc was introduced to four mammalian cell lines. NIH3T3 and HeLa cells transfected with *luc*$^+$ DNA had significantly higher levels of luciferase activity relative to NIH3T3 and HeLa cells transfected with *luc* DNA (Table 3), while CHO and CV-1 cells transfected with *luc*$^+$ or *luc* DNA had comparable luciferase activity. However, it is unclear what alterations in *luc*$^+$ DNA increased luciferase activity in mammalian cells, and why those alterations did not uniformly increase luciferase activity in all the tested mammalian cells. In contrast, a synthetic *Renilla* luciferase gene of the invention was expressed at significantly higher levels relative to a wild type *Renilla* luciferase gene in NIH3T3, HeLa, CHO and CV-1 cells (Table 10).

Sherf et al. do not teach or suggest that modification of a parent sequence to remove palindromic sequences, restriction endonuclease sites, glycosylation sites, and transcription factor binding sites may introduce other undesirable sequences. Nor do Sherf et al. disclose or suggest replacing at least 25% of the codons in a parent sequence with selected mammalian codons, thereby reducing a large number of transcription factor binding sequences in the parent sequence.

A humanized version of a green fluorescent protein (GFP) gene is disclosed in Zolotukhin et al. in which 88/238 of the codons in the gene were altered (column 13, lines 1-4). Zolotukhin et al. do not disclose or suggest that codon optimization of a parent sequence may introduce undesirable sequences.

WO 97/47358 discloses the preparation of synthetic hepatitis C virus (HCV) genes. In particular, it is disclosed that codons in the corresponding wild-type gene that are not the most commonly employed in humans, are replaced with an optimal codon. If a CG is created by that codon replacement, i.e., the third nucleotide in the replaced codon is C and the first nucleotide in the adjacent codon is G, WO 97/47358 discloses that a different codon is selected based on Table 5 in Lathe et al. (J. Mol. Biol., 183:1 (1985)) (page 17). Once all codon replacements are made, it is disclosed that the codon optimized gene is inspected for undesired sequences such as ATTTA sequences, inadvertent creation of intron splice sites, and unwanted restriction enzyme sites, which are then eliminated by substituting codons (pages 17-18). The bias away from CG residues during codon optimization would reduce overall CG content in the final synthetic sequence unless codon substitution to remove undesired sequences resulted in an increase in CG dinucleotides in adjacent codons (thus defeating the reasoning behind avoiding CGs in adjacent codons). In that regard, note that the synthetic click beetle and *Renilla* luciferase genes described in the Examples had increased CG content relative to the respective parent sequence.

WO 97/47358 provides no details of the sequence of any undesirable sites including intron splice sites which are to be eliminated or how to substitute codons to remove ATTTA sequences, polyA sequences, splicing sites and A or T strings > 4. Further, there is no recognition in WO 97/47358 that codon optimization may introduce transcription factor binding sequences or that transcription factor binding sequences may be removed from sequences.

Pan et al. describe a synthetic gene derived from the merozoite surface protein-1 gene (*msp*-1) of *Plasmodium falciparum*. The synthetic gene was prepared by first back translating the corresponding wild type gene using random (not preferred) human codon replacement, choosing one master sequence, and then modifying the master synthetic sequence via alternate codon replacement to eliminate sequences that might be detrimental to efficient transcription and translation, i.e., endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines (page 1095). Notably Pan et al. did not seek to eliminate transcription factor binding sequences in *msp*-1 and did not recognize that codon optimization may introduce transcription factor binding sequences. Nor does Pan et al. disclose the sequences for prokaryotic promoters, poly(A) signals, or exon-intron boundaries that were to be identified for removal.

Cornelissen et al. relate that DNA encoding a Bt crystal protein (insect protein) is modified by changing A and T sequences to G and C sequences encoding the same amino acids (Abstract), in a region that would otherwise have a low percentage of RNA polymerase II compared to an adjacent, upstream region (page 6), to improve expression in plant cells. It is disclosed that *in vitro* binding assays with proteins from tobacco nuclei showed binding in a region 700 to 1000 nucleotides downstream of the transcriptional start site (page 10). It is disclosed that, in particular, a 29 bp region in a 268 bp fragment between positions 733 and 1000 in the Bt protein coding sequence has two sequences which may reduce elongation efficiency. A 326 bp fragment in the Bt protein coding region was replaced with one having 59 codons modified by changing A and T sequences to G and C sequences (page 12). Cornelissen et al. do not disclose or suggest modifying reporter sequences, e.g., luciferase sequences, or recognize that codon replacement may introduce undesirable sites.

Hey et al. disclose altering codons in storage proteins to yield sink protein nucleic acid sequences that have Trp codons for Phe codons (to increase the nutritional value of seed) and also have a reduction in splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues (column 2, lines 53-67 and column 6, lines 22-25). It is disclosed that a sink protein sequence was back translated and codons preferred in maize introduced (column 10, lines 55-66), and restriction

enzymes sites, splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues replaced with second or third choice codons (column 11, lines 2-15). Hey et al. do not disclose or suggest modifying reporter sequences, or recognize that mammalian codon replacement introduces mammalian transcription factor binding sites.

WO 99/14336 discloses thermostable beetle luciferases and a method to prepare those luciferases. It is disclosed that the thermostable beetle luciferases have a plurality of amino acid substitutions relative to a wild-type beetle luciferase, and may be prepared by iterative mutagenesis and selection methods.

In order for the Examiner to establish a *prima facie* case of obviousness, three base criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on Applicant's disclosure. M.P.E.P. § 2142 (citing In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d (BNA) 1438 (Fed. Cir. 1991)).

The combination of references does not disclose or suggest Applicant's invention as each reference discloses a different way to modify the coding sequence of a different gene, i.e., viral genes, a gene from a parasite associated with malaria, an insect toxin gene, a storage protein gene, or a reporter gene, to increase expression. That is, Zolotukhin et al. disclose codon modification alone to codons employed more frequently in one organism generally throughout a green fluorescent protein gene, Sherf et al. disclose limited and targeted modification (modifications in 20 regions of 6 to 30 bp) of a firefly luciferase sequence to introduce or remove cloning sites, alter insect codons to mammalian codons, and to remove post-translation modification sites, secondary structure, and transcription factor binding sites, Cornelissen et al. disclose targeted modification of a toxin gene to alter *Bacillus* codons to remove sequences that may alter elongation efficiency, e.g., by replacing A and T sequences with G and C sequences, WO 97/47358 describes codon replacement to more commonly employed codons combined with

further codon substitution to remove CG residue in adjacent codons, and then inspection for ATTTA sequences, intron splice sites, and unwanted restriction enzyme sites, Hey et al. describe codon replacement in storage protein genes to maize codons and reducing restriction enzyme sites, splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets, and blocks of G or C residues, Pan et al. disclose random human codon replacement yielding a population of synthetic sequences with codon substitutions, choosing one master synthetic sequence, and then modifying the master synthetic sequence via alternate codon replacement to eliminate sequences that might be detrimental to efficient transcription and translation, i.e., endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines, and WO 99/14336 discloses modified thermostable beetle luciferases prepared by mutagenesis leading to a plurality of amino acid substitutions, and selection for one or more phenotypes.

Thus, while there is a general teaching in the combination of cited documents to alter codons and/or remove certain undesired sequences in a selected sequence, none of the cited documents teaches or suggests that codon alterations, to prepare a sequence with codons employed more frequently in an evolutionarily divergent organism optionally in conjunction with removal of restriction enzyme sites, ATTTA sequences, splice sites, polyA sites, A or T strings, CG dinucleotides in adjacent codons, prokaryotic promoters, inverted repeats and prokaryotic factor-independent RNA polymerase terminators, may create transcription factor binding sites. Moreover, none of the cited documents discloses or suggests removal of transcription factor binding sites from a codon optimized gene.

And although one of skill in the art in possession of the cited documents may be motivated to alter the codons of a particular sequence, there is no direction in the combination of cited documents which yields Applicant's invention. It is only with hindsight, i.e., with knowledge of Applicant's invention, that one of skill in the art, picking and choosing from the cited documents, is directed to Applicant's invention. That is, to arrive at Applicant's invention, one of skill in the art in possession of the cited art, would chose to modify a reporter gene (Sherf et al., Zolotukhin et al. and WO 99/14336), rather than a non-reporter gene (WO 97/47358, Cornelissen et al., Hey et al., and Pan et al.), by codon replacement of an entire opening reading

frame (Zolotukhin et al., WO 97/47358, Hey et al. and Pan et al.) rather than by alterations in a portion of an open reading frame (Sherf et al. and Cornelissen et al.) or mutagenesis and selection (WO 99/14336), subsequent additional directed alterations to the nucleotide sequence to remove undesired sequences introduced by codon replacement (WO 97/47358, Hey et al. and Pan et al.) rather than a lack of substantive subsequent additional directed alterations to the nucleotide sequence to remove undesired sequences introduced by codon replacement or concurrently with other alterations, or via selection (Sherf et al., Zolotukhin et al., Cornelissen et al., and WO 99/14336), where codons are replaced with preferred mammalian codons (Sherf et al., Zolotukhin et al., and WO 97/47358), rather than random codon replacement (Pan et al.), plant codons (Hey et al.), G and C residues (Cornelissen et al.), or codons selected for by screening for one or more phenotypes (WO 99/14336).

Moreover, to arrive at Applicant's invention, one of skill in the art in possession of the cited documents would choose to identity transcription factor binding sites (Sherf et al. and possibly Cornelissen et al.), promoter sequences (Pan et al.), splice sites (WO 97/47358, Hey et al., and Pan et al.), and polyA sites (Hey et al. and Pan et al.), as sequences that may be removed by codon replacement although Sherf et al. teach removal of internal palindromic sequences, restriction endonuclease sites, glycosylation sites, and transcription factor binding sites, WO 97/47358 disclose removing ATTTA sequences, inadvertent creation of intron splice sites, and unwanted restriction enzyme sites, Hey et al. disclose removing splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets, and blocks of G or C residue, box sequences, Pan et al. disclose removing endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines, and Cornelissen et al. modify by changing A and T sequences to G and C sequences, and Zolotukhin et al. and WO 99/14336 do not mention removal of a set of specific regulatory sequences in a codon modified coding region.

Further, none of the cited documents discloses or suggests the use of software to identify particular regulatory sites, such as mammalian transcription factor binding sequences, in a database of transcription factor binding sequences.

The Examiner is requested to consider that after codon optimization in conjunction with removal of non-transcription factor binding sites in click beetle and *Renilla* luciferase nucleotide

sequences, Applicant identified about 100 and about 60 transcription factor binding sequences, respectively. Further codon replacement to remove those sequences yielded synthetic click beetle and *Renilla* luciferase sequences with about 50 and about 20 new transcription factor binding sites, respectively, i.e., they were introduced by codon replacement (Examples 1 and 3). The vast majority of the introduced sequences were subsequently removed to yield a synthetic nucleic acid molecule of the invention.

While the cited documents may provide the motivation to repeat the alterations disclosed therein in a different gene, as there is not teaching or suggestion in the cited documents alone or in combination of Applicant's invention, those documents do not provide the motivation to arrive at Applicant's invention.

Further, one of ordinary skill in the art in possession of the cited art would have no reasonable expectation that any particular set of changes may improve activity in a gene that is to be expressed in a highly evolutionarily distinct cell. For instance, an increase in codon substitutions and a decrease in RNA destabilization sequences in a synthetic gene do not necessarily improve the transcriptional characteristics of the synthetic gene relative to the reference gene. In addition, it is unclear what changes to the HCV genes (WO 97/47358), *msp*-1 gene (Pan et al.) or *luc* (Sherf et al.) sequence result in improved activity in a heterologous host and why replacement of codons in *luc* with codons preferred in mammals and other alterations which resulted in luc^+ did not improve luciferase activity in all mammalian cells which expressed Luc^+.

Accordingly, withdrawal of the § 103 rejections is respectfully requested.

The Nonstatutory Double Patenting Rejection

Claims 1, 3-6, 15, 20-21, 24-39, 41-45, 60, 81, 86, and 89-94 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-50 and 58-60 of copending application Serial No. 10/314,827. This rejection is respectfully traversed.

Claims 1-50 and 58-60 in U.S. published application 20030157643 (assigned Serial No. 10/314,827) are directed to synthetic nucleic acid molecules for a fluorescent polypeptide having a codon composition differing at more than 25% of the codons from a parent nucleic acid

sequence encoding a fluorescent polypeptide, wherein the synthetic nucleic acid molecule has at least 3-fold fewer transcription regulatory sequences relative to the average number of such sequences in the parent nucleic acid sequence, a vector having the synthetic nucleic acid molecules, an expression vector having a backbone with at least 3 fold fewer transcriptional regulatory sequences, a host cell or kit with the expression vector, or a polynucleotide that hybridizes to certain synthetic nucleic acid molecules for fluorescent polypeptides.

Claims 1, 3-6, 15, 20-21, 24-39, 41-45, 60, 81, 86, and 89-94 in the present application are directed to synthetic nucleic acid molecules for chloramphenicol acetyltransferase, *Renilla* luciferase, beetle luciferase, beta-lactamase, beta-glucuronidase or beta-galactosidase.

Therefore, withdrawal of the nonstatutory double patenting rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney at (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop Amendment, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 19 day of June, 2006.

JONATHAN FERGUSON _____

Name Signature